MEASURING VASCULAR PROPERTIES

USING INTRINSIC MRI CONTRAST

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This summary will focus on approaches to study vascular structure and function based on susceptibility differences between blood and surrounding tissue – arising primarily from the oxygenation state of blood. This type of contrast, widely used for studies of brain function, is referred to as BOLD (blood oxygen level dependent) contrast [1] Measurements of this type of contrast in tumors have important clinical and research applications. I will discuss the methods used to make these measurements, and the potential applications of the measurements. Since BOLD MRI measures changes in the water signal lineshape, I will emphasize advantage of high spectral and spatial resolution imaging methods for accurate measurement of BOLD contrast changes, and I will discuss spectrally inhomogeneous changes in the water resonance in small voxels due to changes in tumor oxygenation.

How does BOLD MRI compare with other methods for measuring pO_2? Measurements of tumor oxygenation and changes in tumor oxygenation due to therapy are crucial to an understanding of tumor physiology and response to therapy. Traditionally, quantitative oxygen measurements have been made using oxygen electrodes. In addition a variety of spectroscopic methods have been used. Some of these are based on the use of fluorescent and phosphorescent probes that are sensitive to oxygen tension. Others are based on spectroscopic differences between deoxyhemoglobin and oxyhemoglobin. Although these methods offer some advantages none of them are used routinely and their invasiveness and/or poor spatial resolution has encouraged increased emphasis on the development of Magnetic Resonance Imaging methods that are sensitive to oxygen tension.

Some Magnetic Resonance methods are quantitative, directly measure tumor pO_2, and may allow identification of hypoxic tumor regions and accurate measurement of changes in pO_2 during therapy. A very promising method is based on ^{19}F MRI of signals from perfluorocarbons which are administered either IV [2], or intratumorally [3]. The T_1 of the fluorine nucleus in these molecules is sensitive to local oxygen tension, and since relatively large amounts of fluorine containing molecules can be injected, images with reasonable spatial resolution can be obtained [4, 5]. Another approach is to detect ESR signals from nitroxide spin labels [6]and other probes [7]; the linewidth of these signals is very sensitive to local pO_2. ESR from spin labels measured at low frequencies can provide relatively high resolution images *in vivo* [6]. The ^{19}F MRI and ESR methods have become

very valuable for assessment of pO2 in animal tumor models, but are not at present approved for use in humans.[7]

Applications of BOLD MRI in Cancer: BOLD contrast provides a practical proton MR imaging method that is sensitive to changes in blood oxygenation, and in some contexts, absolute oxygen levels. The large paramagnetic susceptibility of deoxyhemoglobin generates large magnetic field gradients in and around blood vessels which effect the linewidth of the water proton signal as well as T_2. Quantitative, absolute measurements of tumor blood oxygen level using BOLD MRI are problematic, except in large veins, because there are many other important influences on the water proton signal. For example, the water resonance is strongly influenced by iron deposits, calcifications, and interfaces between bone and tissue or, to a lesser extension, different types of tissue. However, BOLD MRI can easily provide a qualitative indication of changes in tumor oxygenation. Equipment used to obtain BOLD MRI measurements is widely available both for clinical measurements in cancer patients and for studies of animal models. As a result, BOLD MRI may be rapidly applied to guide treatment of cancer patients and design of new therapies.

An important example of this is the use of BOLD MRI to evaluate the effects of treatments which are used to increase tumor oxygenation (TOX's), so that radiosensitivity is increased. A number of laboratories have reported large and reproducible increases in T_2^* in a number of rodent tumor models during inhalation of pure oxygen and carbogen [8-11]. In at least one tumor model, the R3230 mammary adenocarcinoma growing in Fisher rats, changes in T_2* during TOX treatments averaged over the whole tumor were strongly correlated with changes measured with oxygen microelectrode [12]. In the same tumor model – changes in tumor oxygenation during carbogen breathing measured from the T_1 of the ^{19}F nucleus in a perfluorocarbon emulsion were compared with changes in water proton T_2* [13]. The ^{19}F and ^{1}H measurements of changes in oxygenation agreed in 65%±11% of pixels (n=14). Agreement was even stronger among pixels where ^{1}H showed increased oxygenation – ^{19}F measurements agreed with ^{1}H measurements in over 79%±11% of these pixels. Similarly, there was strong agreement between the two modalities in pixels where ^{19}F reported no change in pO_2; ^{1}H also showed no changes in 76%±18% of these pixels. In BA1112 tumors, BOLD MRI was able to correctly rank the effects of three tumor oxygenating treatments (carbogen alone, perfluorocarbon (PFC) emulsion injected I.V., and the combination of carbogen and PFC) on tumor hypoxic fraction as measured using classical radiation biology methods [14]. This experimental evidence supports the hypothesis that changes in T_2* reflect changes in the oxygen saturation of blood in tumors caused by tumor oxygenating treatments – and that this is strongly related to changes in extravascular pO_2 and hypoxic fraction.

A more general application of BOLD MRI in tumors would be its use to make absolute measurements of blood oxygenation and thus detect hypoxic regions and predict response to radiotherapy, as well as other therapies that require oxygen as a co-factor for generation of free radicals. Work of Van Zijl and coworkers [15] demonstrates that accurate measurements of blood oxygen levels can be measured in brain, where there are fairly large blood vessels. In fact, oxygen extraction ratios can be measured based on the

difference in oxygen level in an artery feeding a particular regions and a vein draining the region [15]. However, this is more difficult in tumors where blood vessels tend to be small and tortuous, and where there are many of sources of T_2* relaxation. Comparison of the ^{19}F T_1 in perfluorocarbon emulsions taken up into rodent tumors following I.V. injection with the water proton T_2* did not demonstrate a strong correlation of ^{1}H T_2* with pO_2 [13]. Nevertheless – there does appear to be some sensitivity of T_2* to absolute oxygen levels. A recent paper by Rodriguez et al. [16] suggests that absolute measurements of R_2* correctly predict response of rodent tumors to radiotherapy. This implies that R_2* is sensitive to local pO_2.

BOLD contrast changes during carbogen breathing may provide other important diagnostic information. Thomas et al. [17] find a correlation between changes in BOLD contrast during carbogen breathing and the growth rate of tumors in mice. This may occur because rapidly growing tumors have a high metabolic rate and use oxygen rapidly, leading to unusually low blood oxygenation. Carbogen would then produce a relatively large change in blood oxygenation in these tumors, leading to a large change in T_2*. If this holds true in general it would mean that tumor growth could be measured non-invasively with high spatial resolution, and without the need for prolonged serial measurements. Neeman et al. have used response to carbogen as a measure of the 'maturity of tumor blood vessels' i.e. development of vascular smooth muscle [18]. This may be another indicator of the state of tumor development.

How are BOLD MRI measurements made: A number of methods have been used to image T_2* and changes in T_2* caused by treatments that affect tumor oxygenation. . Heavily T_1 and T_2* weighted gradient echo images acquired at very high spatial resolution are sensitive to both in flow effects and changes in water proton T_2*. This approach, referred to as FLOOD (Flow and Oxygen Level dependent imaging) has demonstrated both blood oxygenation and blood flow changes caused by carbogen and other tumor oxygenating agents [9]. An important feature of these images is that blood vessels feeding and/or draining tumors can be imaged with high resolution (Simon Robinson et al. personal communication) and changes in the blood flow rates through these vessels caused by tumor oxygenating agents are easily visualized. Griffiths et al. [10] used gradient echo MRI to measure effects of carbogen inhalation on tumor oxygenation in human head and neck tumors. Another approach to imaging effects of TOX's has been to use spectroscopic imaging with high spectral and spatial resolution (HiSS) [19-21]. This is done using either conventional spectroscopic imaging (with phase encoding gradients providing spatial resolution [22]) or echo planar spectroscopic imaging [23]. A related approach is to acquire a train of gradient echoes following excitation, and analyze the decay of the resulting FID in the time domain [24]. High resolution spectroscopic information allows separation of the effects of TOX's on T_1, T_2*, and resonance frequency. HiSS is a useful approach for MR imaging in tumors because the water resonance in tumors is inhomogeneously broadened and often has multiple resolvable components with different T_1, T_2*, and or resonance frequency. This spectral inhomogeneity causes blurring and loss of contrast in conventional MR images. HiSS accurately represents each of these components and produces images that approach the quality that would be obtained with a single very narrow water line. Images of different components of the water line (we

have referred to these as 'Fourier Component Images', or FCIs [25]) often show different anatomic features (see below) – for example blood vessels appear in some FCIs but not in others. In addition HiSS detects spectrally inhomogeneous effects of TOX's on the water signal [26], suggesting sub-voxel heterogeneity in the response to TOX's. These effects are not accurately represented in gradient echo images and may be missed completely. However, the price of this added information is that the run time for very high resolution EPSI datasets is longer than for conventional images.

***Fourier component imaging*:** Recent work from this laboratory has suggested the importance of imaging the various Fourier components of the water resonance. Images can be produced with intensity proportional to the amplitudes of the various Fourier Components of the water resonance – referred to here as FCI's. Experimental data from rodent tumors and human breast suggest that the off-peak Fourier components show anatomic features that are different from the features in water peak height images (i.e., images of the Fourier component with greatest amplitude.) In addition, changes in image contrast due to changes in tumor oxygenation are often different in the different Fourier Component images [25, 26]. This suggests that in some tumors, the magnitude and direction of the measured BOLD contrast change depends on which component of the water resonance one chooses to image. In cases where the water line is inhomogeneously broadened, this can make interpretation of BOLD contrast changes complicated and challenging. In order to accurately measure changes in oxygenation with MRI it is important to determine how to interpret changes in the various FCI's – this information is not currently available. At the same time, the information in off-peak Fourier components of the water resonance provides a potentially important opportunity. If contrast and changes in contrast in the various FCI's reflect changes in different subvoxelar environments – thus it may be possible to study different subvoxelar environments – and changes in oxygenation during therapy in these environments.

The morphologic and functional information present in FCI's is, in principle, also present in conventional images. However, in conventional images the features that appear in different frequency components of the water resonance are superimposed in a way that may cause blurring and loss of information. Individual Fourier Component Images may be the most effective way to view this functional and anatomic information – particularly BOLD contrast changes.

Physiologic monitoring: Regardless of the method used to make BOLD MRI measurements, careful attention to anesthesia and physiological monitoring is critical. Effects of tumor oxygenation and changes in oxygenation on T_2* are subtle, and the magnitude of signal changes caused by oxygenating protocols are generally in the range of 1% - 5%. Therefore, physiological and mechanical stability during experiments is essential. Anesthesia protocols have dramatic effects on spatial and temporal patterns of tumor oxygenation and physiological stability in general. In our laboratory we use isoflurane routinely for anesthesia because it has minimal cardiovascular affects. However, isoflurane has significant affects on blood glucose and this can affect tumor metabolism and therefore tumor oxygen. Temperature, blood pressure, heart rate, and respiration should be monitored continuously during BOLD MRI experiments and

adjustments should be made frequently to maximize stability. We find that respiratory gating greatly increases sensitivity to small changes in oxygenation. Cardiac gating is also helpful. In fact, preliminary results obtained in our laboratory suggest that changes in T_2* during the cardiac cycle may provide novel diagnostic information.

Conclusions: BOLD MRI offers that possibility that changes in tumor oxygenation caused by therapeutic intervention can be imaged non-invasively with high spatial resolution. Response to various oxygenation protocols may contain valuable information concerning tumor biology and physiology that may guide the development of improved therapy, and may aid provide Radiologists with important diagnostic information. Absolute measurements of tumor oxygenation with BOLD MRI might have even broader applications. However, absolute oxygenation measurements are more difficult, because of the variety of mechanisms of T_2* relaxation in tumors. In tumors where the water resonance in small voxels is inhomogeneously broadened, high spectral and spatial resolution imaging may be the most accurate way to measure BOLD contrast changes.

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